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# IDENTITY OF THE PHOTOSYSTEM II REACTION CENTER POLYPEPTIDE *

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**A Photosystem-II (PS-II)-enriched chloroplast submembrane fraction has been subjected to non-denaturing gel-electrophoresis. Two chlorophyll *a* (Chl *a*)-binding proteins associated with the core complex were isolated and spectrally characterized. The Chl protein with apparent apoprotein mass of 47 kDa (CP47) displayed a 695 nm fluorescence emission maximum (77 K) and light-induced absorption characteristics indicating the presence of the reaction center Chl, P-680, and its primary electron acceptor, pheophytin. A Chl protein of apparent apoprotein mass of 43 kDa (CP43) displayed a fluorescence emission maximum at 685 nm. We conclude that CP43 serves as an antenna Chl protein and the PS II reaction center is located in CP47.**

## Introduction

Our research is directed at understanding the protein composition and functional activities of individual polypeptides which comprise Photosystem II (PS II). Satoh [1] reported a procedure for the isolation of a PS II core complex in which he attributed a 43 kDa polypeptide to the chlorophyll *a*-binding apoprotein of the PS II reaction center. More recently, we resolved another protein species in the 47–50 kDa size class after reanalysis of this core complex using SDS-polyacrylamide gel electrophoresis in the presence of urea [2,3]. The variability in the apparent molecular weight of the larger polypeptide, and the ability to resolve it from the 43 kDa component, depended upon the urea concentration in the gel. A comparison of the partial papain hydrolysis products of the 43 and 47 kDa proteins in the PS II core complex by SDS-polyacrylamide gel electrophoresis showed that they were dissimilar [3]. Camm and Green [4,5] described another procedure for the isolation of a photochemically active PS II core complex which also contained the 43 and 47 kDa polypeptides. These analyses of isolated, detergent-derived particles complement earlier genetic studies assigning two chlorophyll *a*-binding proteins of 40–50 kDa to the reaction center complex of Photosystem II [6–8].

Chlorophyll fluorescence has frequently been used as an intrinsic membrane probe to study the primary processes of photosynthesis. Three chlorophyll fluorescence emission bands at low temperature (77 K) have typically been observed from the isolated chloroplasts of higher plants and algae at 685, 695 and 710–740 nm. Butler [9] has developed a tripartite model to assign the origin of the fluorescence emission bands to the light-harvesting

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Abbreviations: PS, Photosystem; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; LDS, lithium dodecyl sulfate; LHC, light-harvesting chlorophyll *a*/*b* complex; $LHC_m$, monomeric form of LHC; Pheo, pheophytin; Chl, chlorophyll.

chlorophyll *a/b* complex (LHC) and the PS II and the PS I complexes, respectively. Of interest to the studies described here is the fact that the 695 nm emission band has been assigned as originating directly from the PS II traps (see Refs. 10–13; however, see also Ref. 14).

The highly resolved PS II core complex originally described by Satoh [1] exhibited two chlorophyll fluorescence emission bands (685 and 695 nm) at low temperature, although the presence of *o*-phenanthroline was required to resolve the latter peak [15]. We thought that possibly one of the emission bands originated from a light-harvesting pigment protein and the other from the reaction center pigment protein. This concept was supported by the studies on a similar PS II complex by Larkum and Anderson [16]. They reported changes in the ratio of the 685 to the 695 nm fluorescence band when they altered the particle environment by lipid dilution and aggregation conditions.

The most widely used polyacrylamide gel electrophoresis systems for separating Chl-binding proteins utilize anionic detergents (for example, see Refs. 7,17 and 18). In preliminary studies on the PS II core complex, we found that these procedures caused denaturation of chlorophyll (Chl) proteins such that the 695 nm fluorescing species was lost. The detergent extraction procedure developed by Camm and Green [18], using octyl-β-D-glucopyranoside, was found to be less harsh and we have, therefore, adopted their sample preparation protocol to characterize the Chl *a*-binding proteins of Photosystem II. This has allowed us in this report to assign the origin of the 685 and 695 nm fluorescence emission bands to specific pigment proteins.

## Materials and Methods

Market spinach (50 g) was homogenized in a Waring blender in a volume of 100 ml comprising 0.2 M sorbitol/50 mM Tricine-HCl (pH 7.8)/10 mM $MgCl_2$/5 mM NaCl/5 mM sodium ascorbate/1 mg · $ml^{-1}$ bovine serum albumin. After filtration through eight layers of cheesecloth, the filtrate was sedimented at 1000 × *g* for 5 min in a Sorvall SS-34 rotor and washed once with 2 mM Tris-malic acid (pH 7.0), and centrifuged as above. The washed membranes were then thoroughly dispersed in a small volume of the Tris buffer, and diluted and incubated with 30 mM octyl-β-D-glucopyranoside at a detergent-to-chlorophyll ratio of 40 : 1 [18]. Chlorophyll was determined by the method of Arnon [19]. After dark incubation at room temperature for 30 min, the suspension was centrifuged at 110 000 × *g* for 30 min. The supernatant was concentrated against solid sucrose and subjected to lithium dodecyl sulfate (LDS)-polyacrylamide gel electrophoresis at 4°C using the Laemlli buffer system with LDS (1% w/v) rather than sodium dodecyl sulfate (SDS) [6,17]. Gel slices containing the pigmented proteins were excised from the slab gel (maintained on ice in low light) and examined immediately. Absorption spectra were obtained with a Hitachi-110 split-beam spectrophotometer. Low-temperature fluorescence spectra of gel slices were obtained as in Ref. 3 using a SLM System 4000 spectrofluorometer. The fluorescence photograph of an intact pigment protein gel was obtained with a Polaroid type 47-3000 Land roll film during illumination of the LDS-polyacrylamide gel with a long-wavelength ultraviolet-emitting Spectroline Transilluminator, model TR 302.

Room-temperature fluorescence yield changes and light-induced absorbance changes were measured at the C.F. Kettering Laboratory, Yellow Springs, OH, using the diode array rapid-scanning spectrometer (DARSS Tracor Northern 1710), and a phosphoroscope-type illumination chamber as described in Ref. 20. Gel slices were sandwiched within a lucite template and placed in a 1 × 1 cm cuvette. The cuvette front surface was masked with black tape to define the cross-section for the incident measuring beam. The samples were immersed in a solution comprising 0.1 M Tricine-NaOH (pH 8.9)/5 mM $MgCl_2$.

## Results and Discussion

Electrophoresis by non-denaturing LDS-polyacrylamide gel electrophoresis of the PS II-enriched fraction obtained by octyl-glucopyranoside extraction led to the resolution of four major pigmented zones including the free-pigment as previously shown by Camm and Green [18,21] (see Fig. 1, lane 1). The uppermost band is labelled

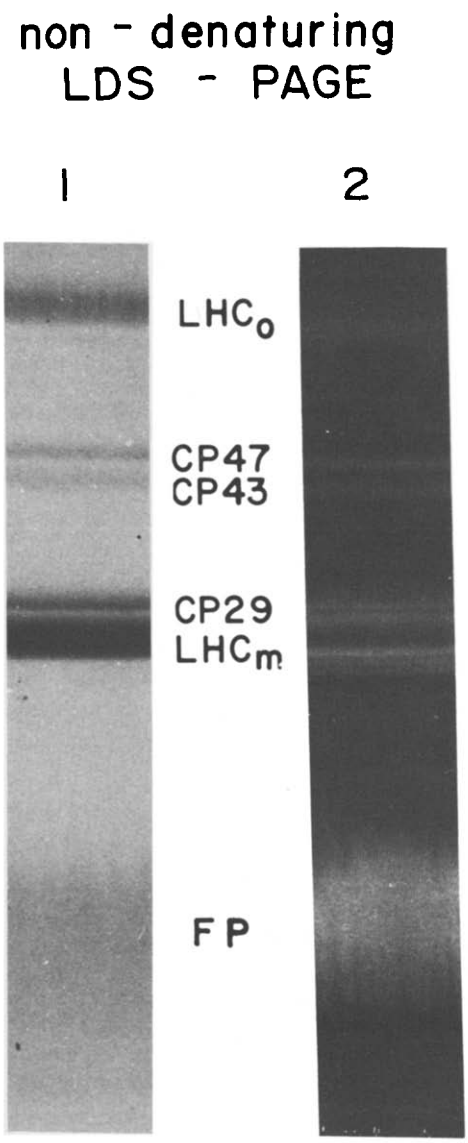


Fig. 1. Photograph of the chlorophyll protein bands (lane 1) separated by subjecting the PS-II-enriched octyl-β-D-glucopyranoside extract to non-denaturing LDS-polyacrylamide gel electrophoresis (PAGE). The reaction center chlorophyll *a*-binding proteins are designated CP47 and CP43. Lane 2 is a fluorescence photograph obtained by ultraviolet illumination of the acrylamide gel in lane 1.

$LHC_o$, i.e., an oligomeric form of the light-harvesting chlorophyll *a/b* complex (LHC) based upon a comparison of its absorption and first derivative spectra (not shown) to that of the purified LHC [22,23]. The next pigmented zone in the gel was resolved into the two chlorophyll *a*-binding proteins, CP47 and CP43, using the previous nomenclature of Camm and Green [18]. These have been renamed CP a-1 and CP a-2, respectively by Camm and Green [5]. They are concluded to be analogous to the putative PS II reaction center chlorophyll *a*-binding proteins observed in *Chlamydomonas* [7] and barley (Ref. 6 but see also Ref. 8). The major pigment protein migrating more rapidly than the CP47 and CP43 bands in our gels can be assigned to a monomeric form of the $LHC_m$ [see 18,21]. A chlorophyll *b*-containing complex migrating slightly slower than the $LHC_m$ has been referred to as CP29 [18,22]. The band migrating at the gel front is the free pigment. A photograph of the fluorescence emitted by the chlorophyll proteins in this gel system is shown in lane 2 of Fig. 1.

Gel segments containing either the CP47 or CP43 complex were excised and directly characterized. The absorption spectra for both components showed a peak maximum near 670 nm in the red region of their spectra indicative of the presence of chlorophyll *a* (see Fig. 4).

By low-temperature fluorescence spectroscopy (77 K), we found that CP47 exhibited a fluorescence emission band with a maximum at 695 nm when excited at 440 nm, whereas the emission of the CP43 complex peaked at 685 nm (Fig. 2). The emission peaks of samples which were allowed to age at room temperature were often blue-shifted to

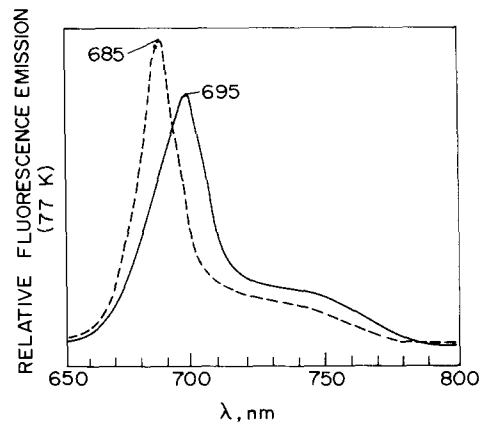


Fig. 2. Low-temperature (77 K) chlorophyll fluorescence emission obtained for the excised CP47 (———) and CP43 (- - - -) components. The emission band for the CP47 component is at 695 nm, whereas that for the CP43 component is at 685 nm. The pigment complexes were excited with 440 nm light.

wavelengths near 680 nm, as previously reported [18,21,24], indicating the extreme lability of the complexes following electrophoresis. Low-temperature (approx. 4°C) and low-power (2–3 W) conditions during electrophoresis appeared to optimize retention of the 685 and 695 nm emission bands in our experiments. Fluorescence excitation spectra for both CP47 and CP43 were found to be essentially identical, with a peak in the Soret region near 440 nm, further confirming the Chl *a* nature of these complexes (see Ref. 34).

To verify that the 685 and 695 nm fluorescence emission bands were due to separate individual polypeptides, the excised CP47 and CP43 pigment proteins were subjected to reelectrophoresis under denaturing conditions. Each was resolved into a single major polypeptide with minimal cross-contamination (see Fig. 3). In total, these results are the first report of individual Chl-binding proteins giving rise separately to the 685 and 695 nm fluorescence emission bands (see also Ref. 33 and 34).

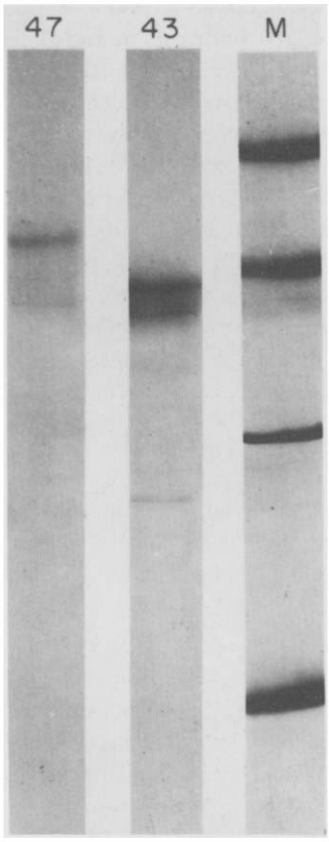


Fig. 3. Excised slices of CP47 and CP43 subjected to electrophoresis under denaturing conditions. Lane 1 contains CP47; lane 2, CP43; lane 3, the molecular weight markers (bovine serum albumin (68 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa) and cytochrome *c* (12.4 kDa)).

Recent studies have suggested that pheophytin acts as an intermediate electron carrier between P-680 and Q [25–27]. In addition, Breton [28] has postulated that the 695 nm low-temperature fluorescence emission band arises from the deactivation of the excited state of the PS II 'primary' acceptor, pheophytin. The absorption spectra of both CP47 and CP43 gave little indication of the enrichment of pheophytin (see Fig. 4 and Ref. 34), i.e., not more than 1–2 per 50 chlorophyll *a* molecules, which was an upper estimate obtained for the PS II core complex preparations [3]. The presence of photochemically active pheophytin was monitored via absorption changes at 685 nm for the crude glucopyranoside extracts of the thylakoid membranes, and the CP47 and CP43 components excised from the gels. This wavelength was chosen, since it represents a peak maximum associated with the photoreduction of pheophytin [29,30]. The kinetic results of one such experiment with the glucopyranoside extract are shown in Fig. 4B and they were used to establish the conditions for the diode array rapid-scanning spectrophotometer analyses; see below. The kinetic analysis indicated that a largely irreversible photobleaching (denoted $\Delta A_2$) and a small reversible absorbance change (denoted $\Delta A_2$) occurred upon illumination of any of the samples. Maximal extent of the reversible absorption change with the glucopyranoside extract required the presence of $NH_2OH$. Dithionite (2 mg/ml) and $NH_2OH$ (20 mM) were routinely added to optimize the reversible component which was kinetically slower with the gel materials.

Evaluation of the nature of the $\Delta A_1$ and $\Delta A_2$ components in the purified pigment proteins (CP47 and CP43) required rapid analysis as a function of wavelength. This was accomplished by using the diode array rapid-scanning spectrophotometer. The diode array rapid-scanning spectrophotometer analysis with appropriate gating at the detector allowed us to obtain separate difference spectra for these absorption changes (see Fig. 4). In the case of CP43, $\Delta A_2$ for this pigment protein was resolved to have an absorption maximum near 667

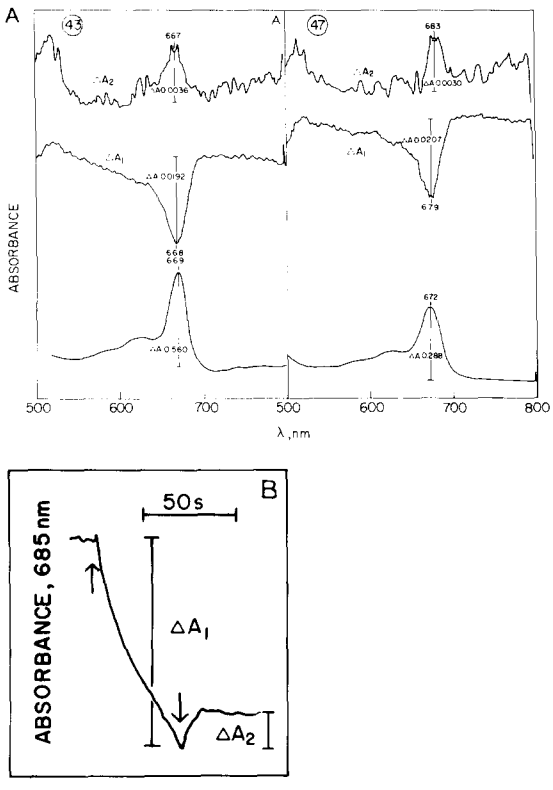


Fig. 4. (A) Diode array rapid-scanning spectrometer analysis in a phosphoroscopic sample chamber of the CP47 and CP43 complexes. A kinetic analysis of the complexes obtained in the presence of dithionite (2 mg/ml) and $NH_2OH$ (20 mM) shows a largely irreversible absorbance change denoted by $\Delta A_1$ upon illumination. A small reversible absorbance change is seen upon cessation of illumination and denoted by $\Delta A_2$. Spectral analyses of the kinetic components were examined over a 300 nm range (500–800 nm) with 512 scans. Illumination was provided through a 4-96 Corning filter and a 560 nm cut-on filter. The lowest spectrum of each figure is the 'dark' absorption spectrum of CP43 or CP47. (B) Kinetic analysis of gluco-pyranoside extract.

nm. These peak maxima are similar to that obtained for the absorption spectrum of the CP43 complex (shown in the lower part of Fig. 4A, left half). We interpret these data to indicate that all light-induced absorbance changes in the CP43 sample are due to nonspecific photo-oxidation of the bulk pigments present in this pigment-protein.

The diode array rapid-scanning spectrophotometer analysis of the CP47 pigment protein resolved the largely irreversible light-induced absorption change, $\Delta A_1$, into a spectral component having a peak maximum at 679 nm (see Fig. 4). We interpret this to indicate an irriversible bleaching of the reaction center antenna pigment absorbing maximally at 680 nm. In similar studies with a reaction center preparation from photosynthetic bacteria, Okamura et al. [31] also observed a large irreversible bleaching upon illumination of the reaction center chlorophylls (see also Ref. 32). The difference spectrum of $\Delta A_2$ for CP47 was resolved into a spectral component having a peak maximum at 683 nm (see also Fig. 4). We interpret the spectral change to be due to pheophytin [20,25–27]. Under such conditions, of course, the P-680$^+$ absorbance change would not be observed at the time resolution of the measurement. Thus, the purified CP47 obtained by gel electrophoresis can be identified as the PS II polypeptide containing the components, P-680 and pheophytin, which catalyzes the primary charge separation of PS II. The significance of the absorption change near 520 for both complexes is unknown.

Characterization of variable fluorescence has frequently been used as a tool to analyze PS II electron-transport reactions. We attempted similar analyses with CP47 and CP43. In neither case could a variable component of fluorescence yield be detected (not shown). This observation would be expected either if the preparations were photochemically inactive (as we believe to be true for CP43) or if the stable primary electron acceptor, a plastoquinone called Q, was absent. We believe that the latter conclusion could best explain the lack of a variable fluorescence yield in CP47. Preliminary room-temperature fluorescence data suggest a similar conclusion (data not shown).

## Conclusion

The results presented in this report confirm the association of two chlorophyll *a*-binding proteins with Photosystem II. The larger pigment protein obtained by non-denaturing gel electrophoresis, denoted CP47, displays characteristics of the reaction center (P-680-pheophytin) and exhibits a low-temperature chlorophyll fluorescence emission at 695 nm previously attributed to PS II traps (Refs. 10–13,28 and 34; however, see Ref. 14). The small light-induced reversible absorption changes of the CP47 complex are indicative of pheophytin

reduction. The CP43 component is thus assigned a light-harvesting role in PS II [33,34]. These conclusions are consistent with recent studies of octylglucopyranoside-solubilized PS II particles separated on sucrose gradients and analyzed for electron-transport activity; photochemistry was preferentially associated with complexes containing the CP47 (Refs. 5, 33 and 34 and see also Ref. 35). Hermann et al. [36] have shown that the reaction center apoproteins are coded for by the plastid chromosomes.

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## References

1 Satoh, K. (1979) Biochim. Biophys. Acta 546, 84–92
2 Nakatani, H.Y., Satoh, K., Steinback, K.E. and Arntzen, C.J. (1983) in IUPAC Pesticide Chemistry (Miyamoto, T., ed.), pp. 171–176, Pergamon Press, New York
3 Satoh, K., Nakatani, H.Y., Steinback, K.E., Watson, J. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 142–150
4 Camm, E.L. and Green, B.R. (1982) Plant Physiol. 69, 28 (Supplement)
5 Camm, E.L. and Green, B.R. (1983) Biochim Biophys. Acta 724, 291–193
6 Machold, O., Simpson, D.J. and Moller, B.L. (1979) Carlsberg Res. Comm. 44, 235–254
7 Delepelaire, P. and Chua, N.-H. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 111–115
8 Hayden, D.B. and Hopkins, W.G. (1977) Can. J. Bot. 55, 2525–2529
9 Butler, W.L. (1978) Annu. Rev. Plant Physiol. 29, 345–278
10 Govindjee (1963) in Photosynthetic Mechanisms of Green Plants, Natl. Acad. Sci. Publ. 1145, 318–334, Natl. Res. Council, Washington D.C.
11 Bergeron, J.A. (1963) Natl. Acad. Sci. Natl. Res. Council Publ. 1145, 527–536
12 Butler, W.L. and Kitajima, M. (1979) Biochim. Biophys. Acta 396, 72–83
13 Rijgersberg, C.P., Amesz, J., Thielen, A.P. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473–482
14 Brown, J. (1977) in Bioenergetics of Membranes (Packer, J., Papageorgiou, G.C. and Trebst, A., eds.), pp. 297–304, Elsevier North-Holland, Amsterdam
15 Satoh, K. (1980) FEBS Lett. 110, 53–56
16 Larkum, A.W.D. and Anderson, J.M. (1982) Biochim. Biophys. Acta 679, 410–421
17 Laemlli, U.K. (1970) Nature 227, 680–685
18 Camm, E.L. and Green, B.R. (1980) Plant Physiol. 66, 428–432
19 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
20 Ke, B., Inoue, H., Babcock, G.T., Fang, Z.X. and Dolan, E. (1982) Biochim. Biophys. Acta 682, 297–306
21 Green, B.R., Camm, E.L. and Van Houten, J. (1982) Biochim. Biophys. Acta 681, 248–255
22 Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–263
23 Mullet, J.E., Baldwin, T.O. and Arntzen, C.J. (1981) in Photosynthesis III, Structure and Molecular Organization of the Photosynthetic Apparatus, (Akoyunoglou, G., ed.), pp. 577–582, Balaban International Science Services, Philadelphia, PA
24 Searle, G.F.W., Koehorst, R.B.M., Schaafsma, T.J., Moller, B.L. and Von Wettstein, D. (1981) Carlsberg Res. Commun. 46, 183–194
25 Klimov, V.V., Klevanik, A.V., Shuvalov, V.A. and Krasnovsky, A.A. (1977) FEBS Lett. 82, 183–186
26 Klimov, V.V., Ke, B. and Dolan, E. (1980) FEBS Lett. 118, 123–126
27 Nugent, J.H.A., Moller, B.L. and Evans, M.C.W. (1980) FEBS Lett. 121, 355–357
28 Breton, J. (1982) FEBS Lett. 147, 16–21
29 Klimov, V.V., Dolan, E. and Ke, B. (1980) FEBS Lett. 112, 97–100
30 Ke, B., Klimov, V.V., Dolan, E., Shaw, E.R., Shuvalov, V.A., Parson, W.W., Fajer, J., Davis, M.S. and Forman, A. (1981) in Photosynthesis III. Structure and Molecular Organization of the Photosynthetic Apparatus (Akoyunoglou, G., ed), pp. 815–826, Balaban International Science Services, Phildelphia, PA.
31 Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3491–3495
32 Rutherford, A.W., Mullet, J.E., Paterson, D.R., Robinson, H.H., Arntzen, C.J. and Crofts, A.R. (1981) in Photosynthesis III, Structure and Molecular Organization of the Photosynthetic Apparatus (Akoyunoglou, G., ed.), pp. 919–928, Balaban International Science Services, Philadelpia, PA
33 Nakatani, H.Y. (1983) in The Oxygen-Evolving System of Photosynthesis (Inoue, Y., Murata, N., Crofts, A.R., Renger, G., Govindjee and Satoh, K., eds.), pp. 49–54, Academic Press, Tokyo
34 Nakatani, H.Y., Arntzen, C.J. and Inoue, Y. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. II, pp. 99–102, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, in the press
35 Yamagishi, A. and Katoh, S. (1983) in The Oxygen-Evolving Sytem of Photosynthesis (Inoue, Y., Murata, N., Crofts, A.R., Renger, G., Govindjee and Satoh, K., eds), pp. 39–48, Academic Press, Tokyo
36 Hermann, R.G., Westhoff, P., Alt, J., Bisanz, C., Heinemeyer, W., Olesch, B. and Nelson, N. (1983) in Abstracts of the 6th International Congress on Photosynthesis, Vol. 2, p. 51, Brussels, Aug. 1–6, 1983